
BIOTECHNOLOGY- AND ITS APPLICATIONS

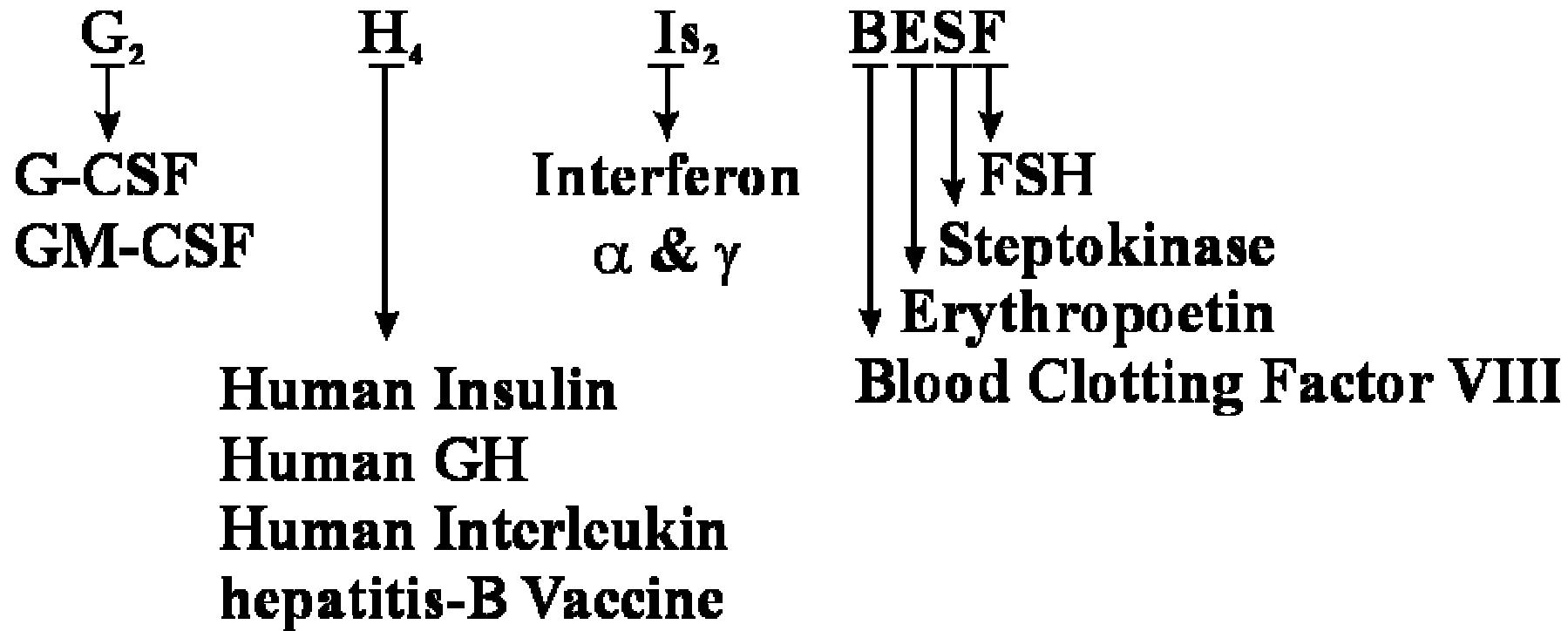
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Biotechnology Application in Medicine

Advantages

1. Mass production
2. Safe and more effective drugs
3. Do not induce unwanted immunological responses as from non human resources
 - ✓ About 30 recombinant therapeutics approved world over
 - ✓ In India, 12 hour marketed

12 Therapeutics Approved in India



Protein

Erythropoietin

Factor VIII

Follicle stimulating hormone

Granulocyte colony stimulating factor

Insulin

Interferon- α

Interferon- γ

Interleukins

Somatotrophin

Tissue plasminogen activator

Used in the Treatment

Anemia

Haemophilia

Infertility treatment

Cancer

Diabetes

Leukemia and other cancers

Cancer, rheumatoid arthritis

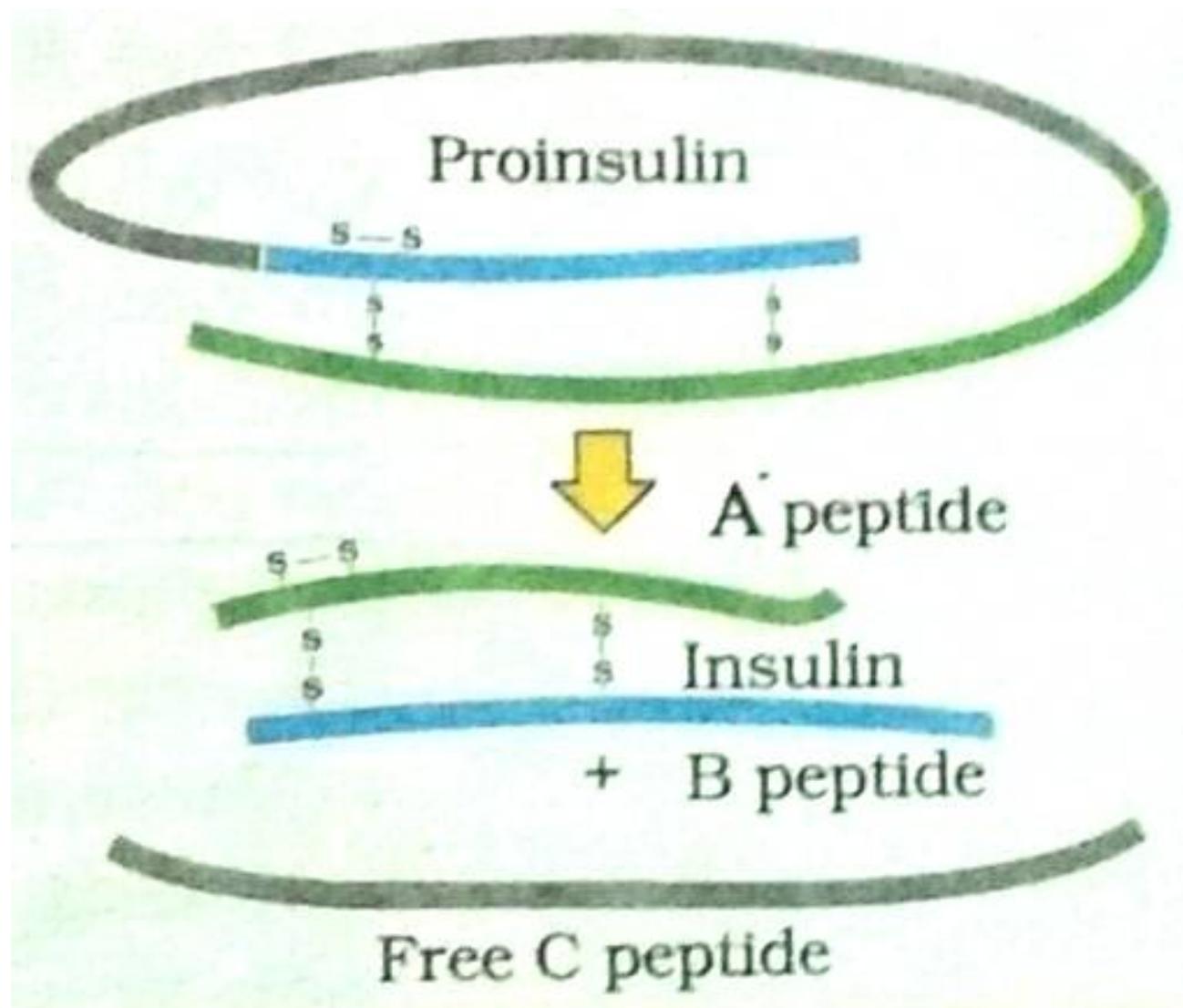
Cancer, immune disorders

Growth disorders

Heart attack

Genetically engineered insulin

- ✓ Earlier extracted from pancreas of slaughtered cattles and pigs (developed allergy /reactions to foreign proteins)
- ✓ Insulin – two short polypeptide chains (A and B)linked together by di sulphide bridges
- ✓ Synthesized as pro hormone in mammals, contains extra C peptide (not present in mature insulin, removed during maturation)
- ✓ Main challenge – getting insulin assembled into mature form
- ✓ In 1983, Eli Lilly (America company) prepared 2 DNA sequences corresponding to A and B chains and introduced them in plasmids of E.coli separately, extracted and combined by creating disulphide bonds
- ✓ Humulin – analoge of human insulin



Maturation of pro-insulin into insulin (simplified)

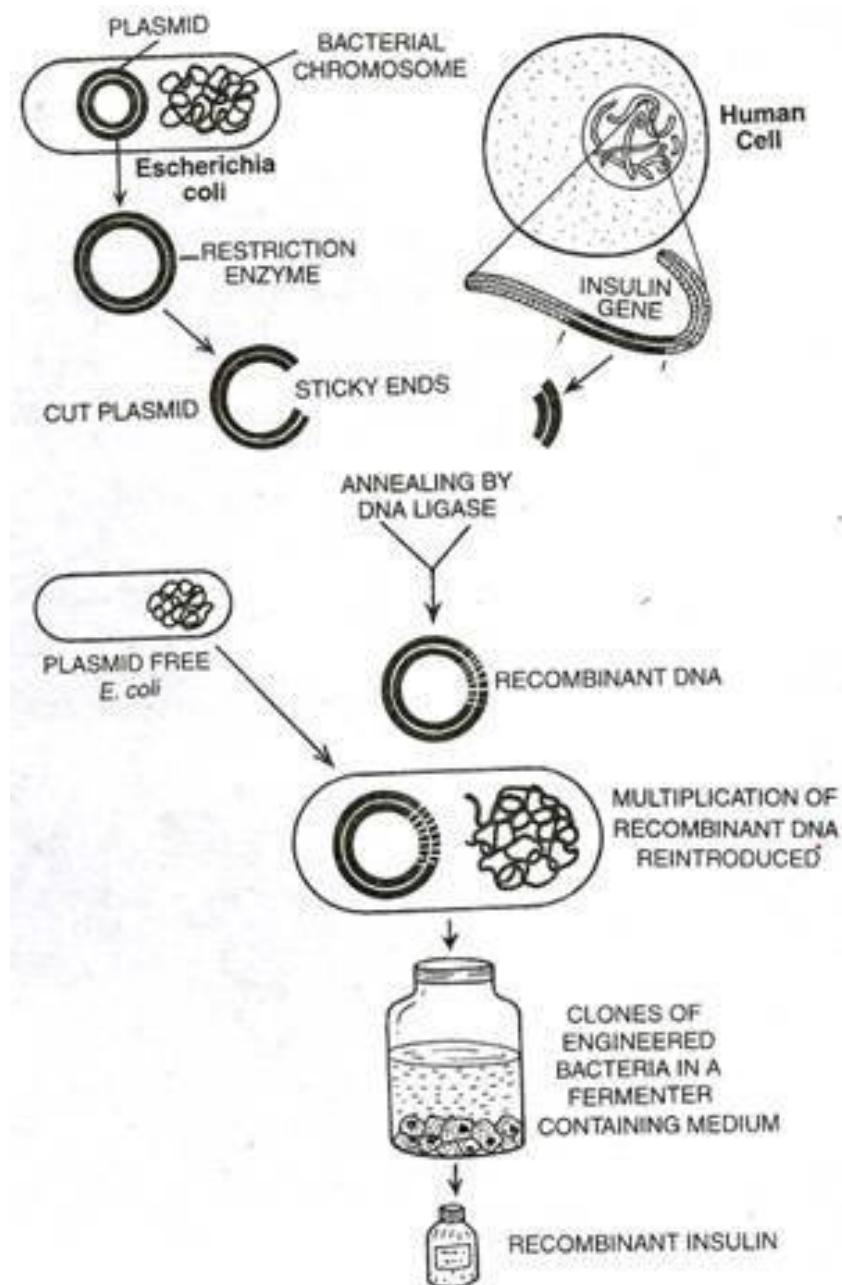


Fig. 12.11. Steps involved in gene transfer for the production of human insulin.

Gene therapy

- ✓ Methods to correct gene defect diagnosed in a child/embryo, genes are inserted into persons cells and tissues to treat a disease
- ✓ Replacement of non-functional gene with a normal gene
- ✓ First clinical gene therapy given in 1990 to four year girl with adenosine deaminase (ADA) deficiency (essential for immune system functioning)
- ✓ SCID (severe combined immuno deficiency) - ADA: deletion of ADA gene

Treatment modalities –

1. Enzyme replacement therapy (ERT – IV injection of ADA),
2. BMT (both not completely curable),
3. Gene therapy – permanent cure if gene isolate from marrow cells producing ADA is introduced into cells at early embryonic stages
 - a) lymphocytes from blood of patient grown in culture outside body
 - b) functional ADA cDNA (using retroviral vector) is introduced into these lymphocytes
 - c) Lymphocytes are subsequently returned to patient

Note : as these cells are not immortal, patient requires periodic infusion of genetically engineered lymphocytes

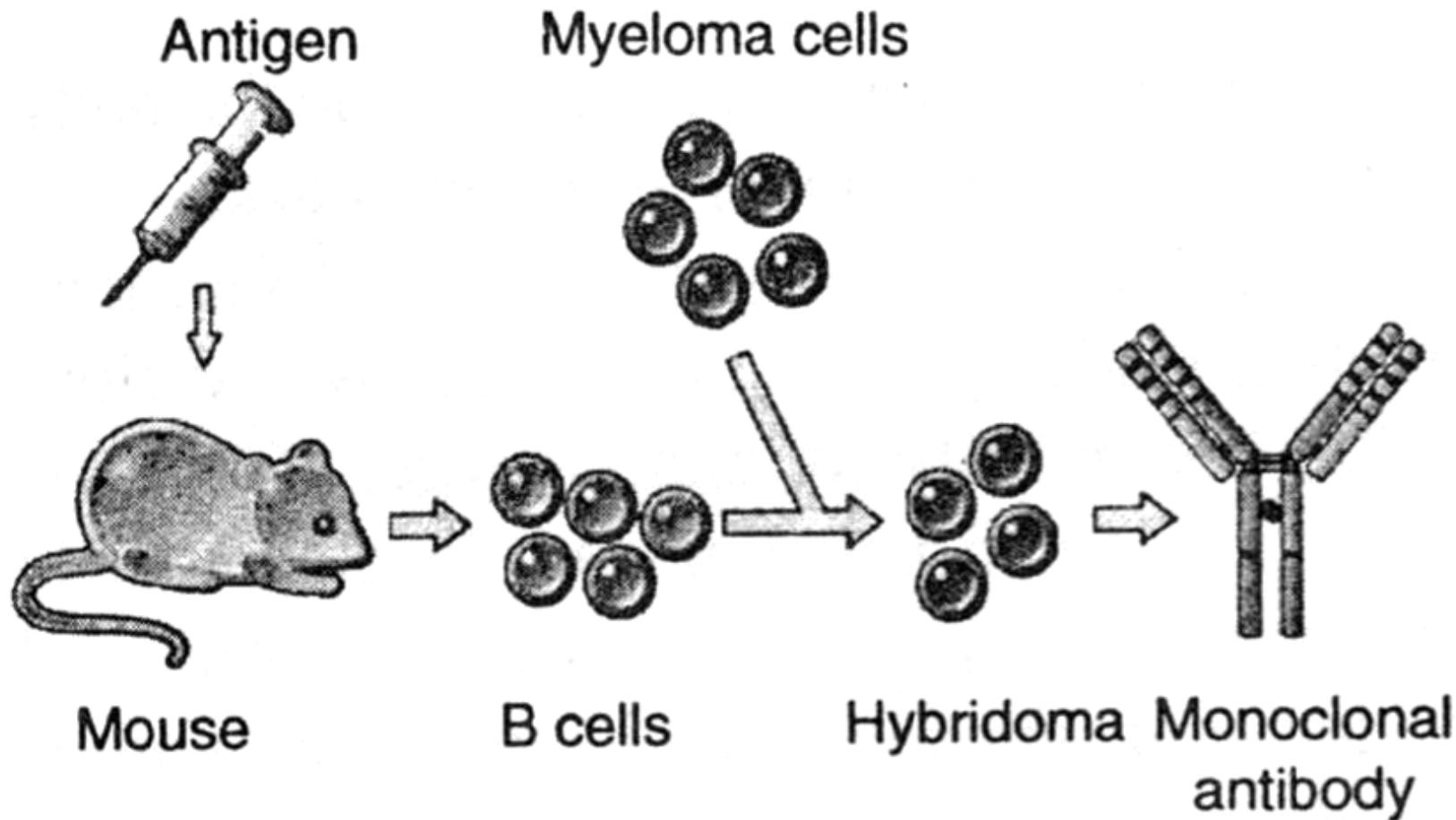


Fig. 25.6 Hybridoma Technique and production of monoclonal antibodies

Trick for Monodonal Antibody -

MA
↓

Aj Bahut Lamba
↓

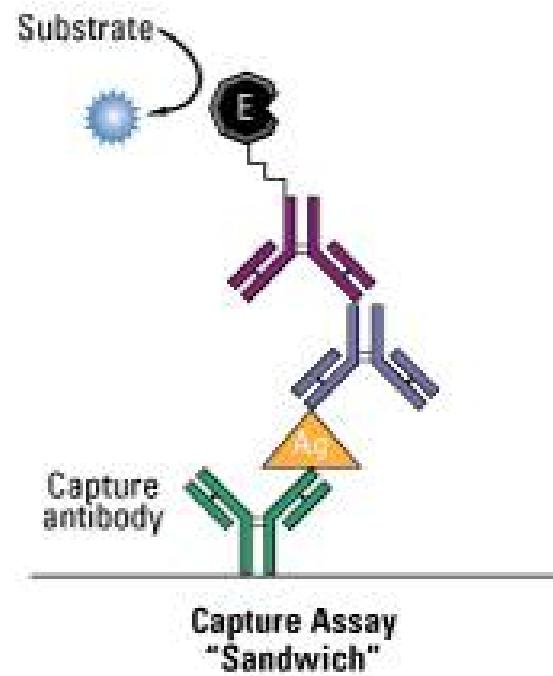
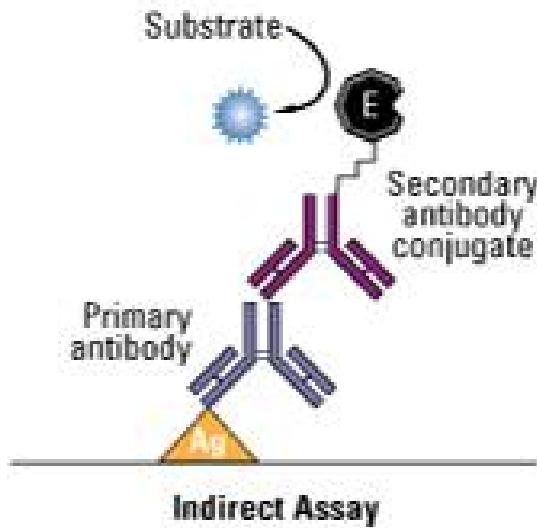
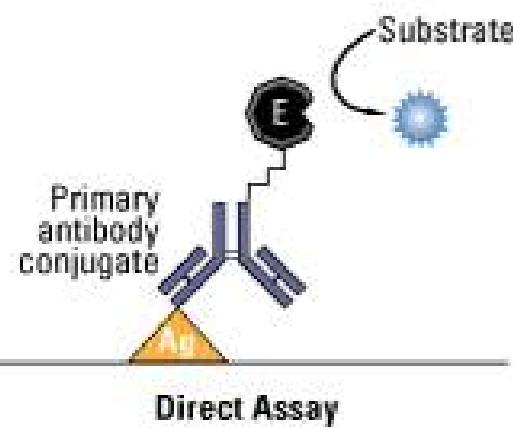
Match
↓

Hua
↓

Monodonal Antibody producing Myeloma cells Hybridoma
Antibody B-Lymphocytes

Molecular diagnosis :

- ✓ Conventional method of diagnosis (serum/urine analysis etc)-early detection not possible
- ✓ Modern methods (rDNA technology/ PCR/ ELISA) - early diagnosis
- ✓ Presense of pathogens normally suspected – when disease symptom produced (pathogen concentration already very high in body)



The most commonly used enzyme include peroxidase and alkaline phosphatase

PCR:

- ✓ Very low concentration of pathogen can be detected by amplification of their nucleic acid by PCR (when symptom not yet visible)
- ✓ Routinely used to detect HIV in suspected AIDS patient
- ✓ Used to detect mutations in genes in suspected cancer patient's
- ✓ Technique to identify many genetic disorder

Autoradiography:

- ✓ Single stranded RNA/DNA, tagged with radio active molecule (probe) is allowed to hybridized to its complimentary DNA in a clone of cells.
- ✓ The clone having mutated gene will not appear on photographic film, because probe will not have complimentarity with mutated gene

ELISA (Enzyme Linked Immuno Sorbent Assay)

- ✓ Principle – antigen, antibody interactions
- ✓ Infection by pathogen can be detected by presence of antigens (proteins, glycoproteins etc.) or by detecting anti bodies synthesized against pathogen
- ✓ Direct elisa – test for antigen presence
- ✓ Indirect elisa – test for anti bodies presence
- ✓ Development of color on addition of chromogenic substrait indicates presence of antigens/ antibodies

Biotechnological applications in agriculture

Three options for increasing food production are-

1. Agro – chemical based agriculture
2. Organic agriculture
3. Genetically engineered crop – based agriculture

- Green revolution – food supply tripled but yet not enough for growing population
- Father of green revolution- Norman Ernest Borlaug
- Resulted from – use of improved crop varieties, better management practices, use of agro chemicals (fertilizer's/ pesticides)
- Hindrances – agro chemicals too expensive, harmful effect on environment, increased yield not possible using conventional breeding
- Overcoming of above problems by GMO(genetically modified organism)

Genetic modification has –

1. Made crops more tolerant to abiotic stress (cold, draught, salt, heat)
2. Reduced reliance on chemical pestisides (pest resistant crop)
3. Reduced post harvest losses
4. Increased efficiency of mineral usage by plant (prevents early exhaustion of soil fertility)
5. Enhanced nutritional value of food, eg. vitamin A enriched rice

Golden rice –

- ✓ Developed by ingo – potrykus and peter beyer
- ✓ Transgenic variety of rice (*oryza sativa*), contains β – carotene (provitamin A-inactive state of vitamin A)
- ✓ Vector used *agrobectirium tumefaciens*
- ✓ Production of hirudin (anticoagulant) from transgenic *brassica napus* seats

Note: GM has been used to create tailor- made plants to supply alternative resources to industries, in the form of starch, fuels and pharmaceuticals.

Production of hirudin from transgenic *Brassica napus* seeds.

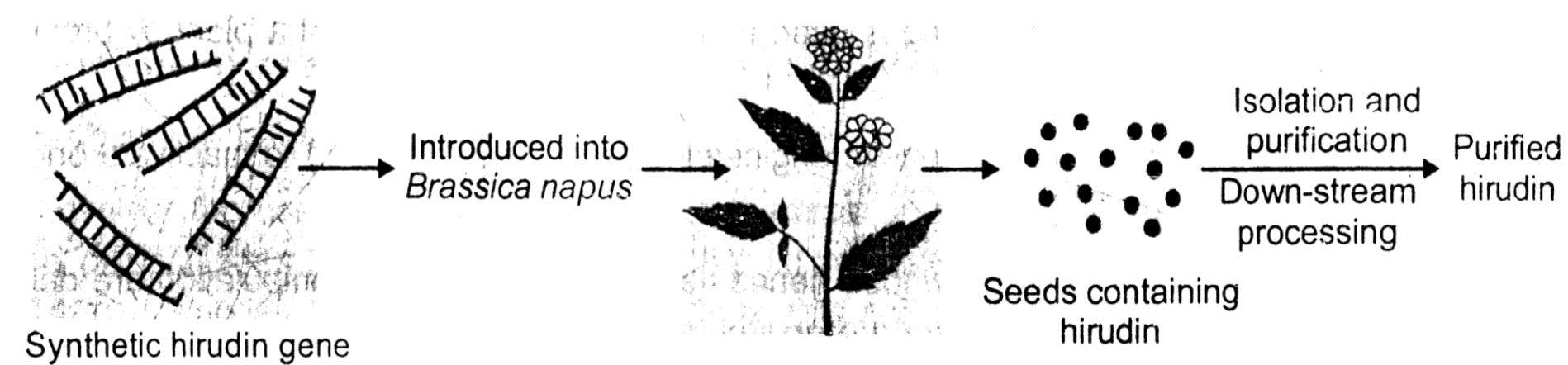
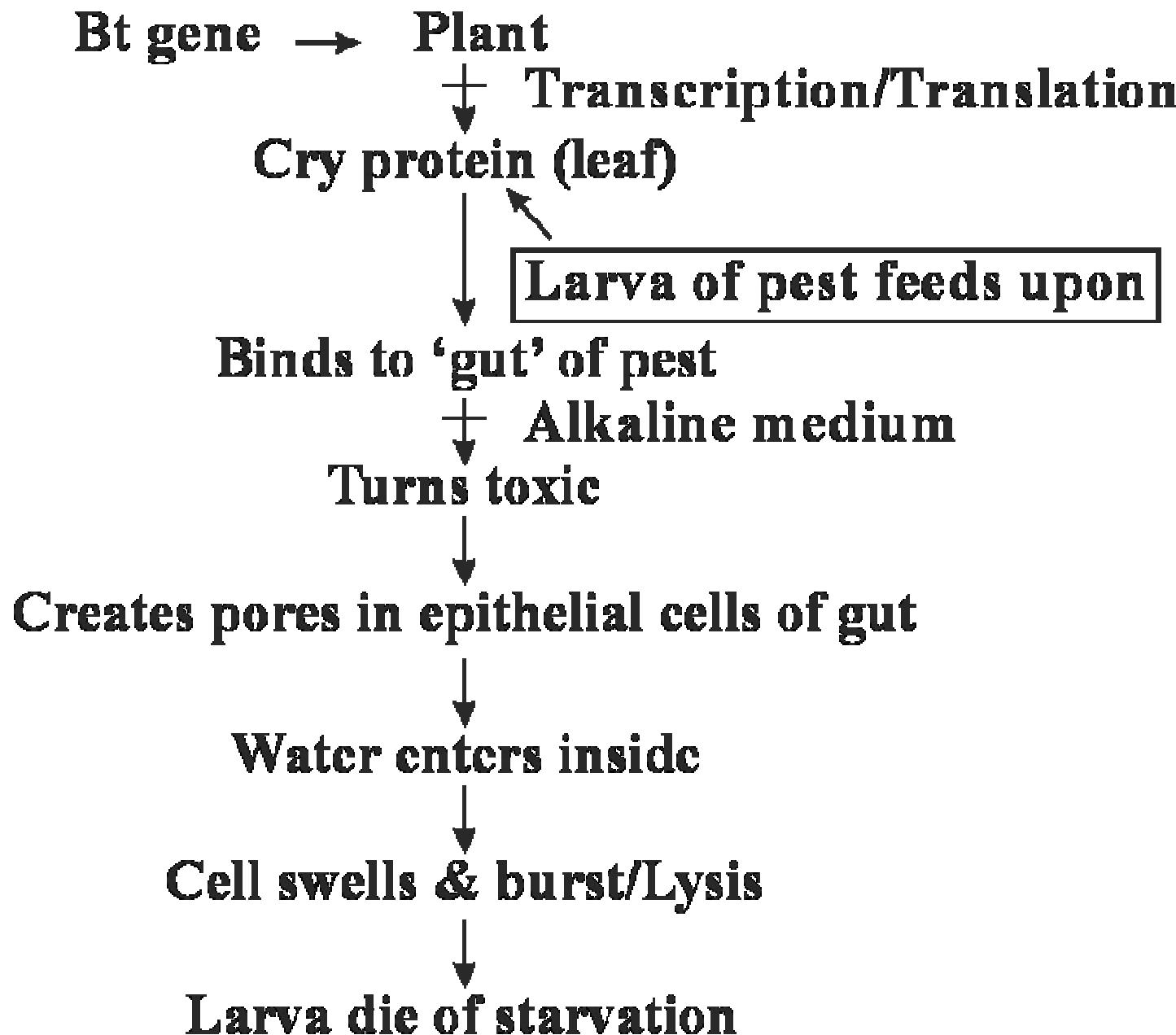
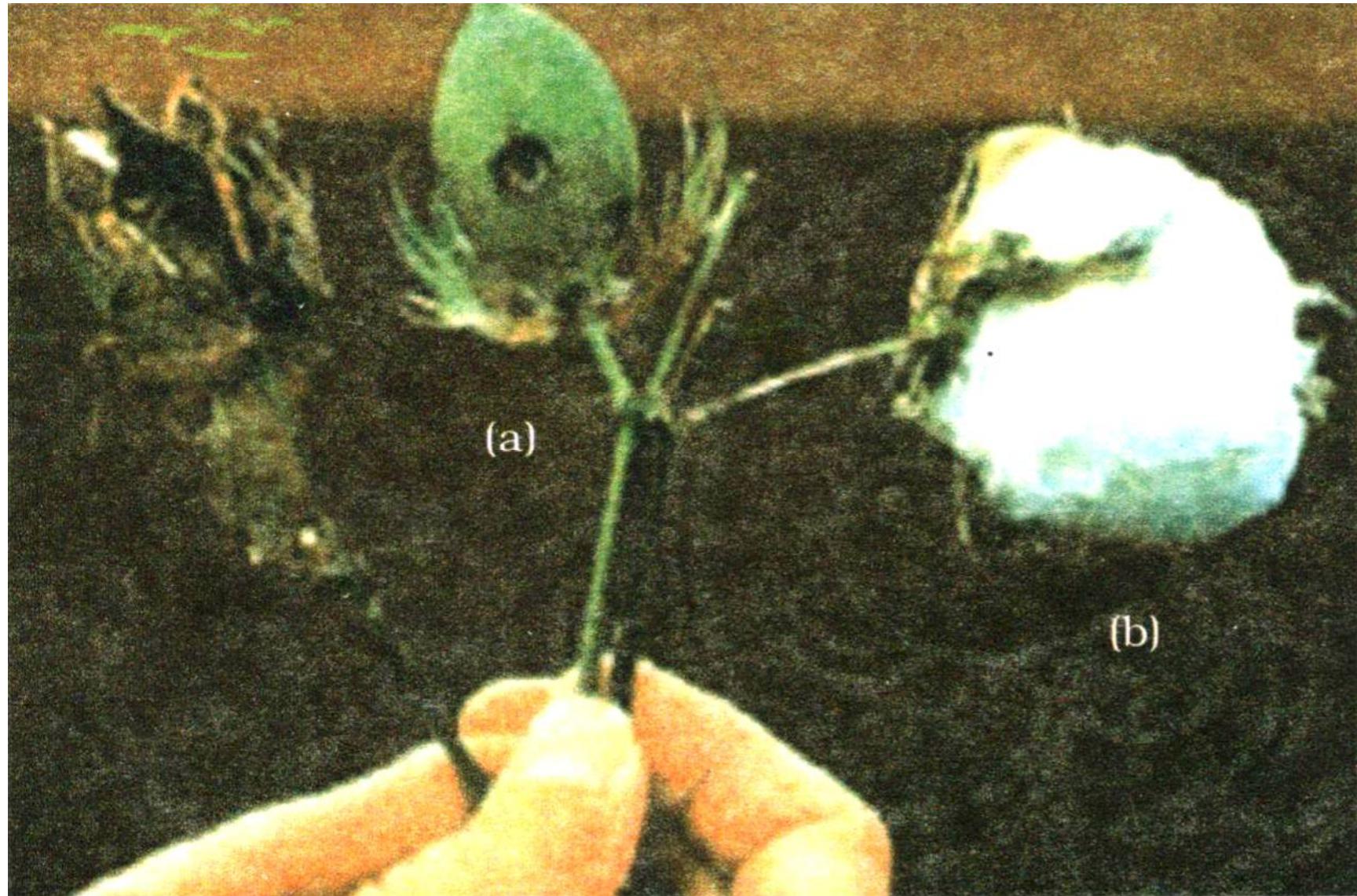


Fig. : Transgenic *Brassica napus*

Bt toxin :-





**Cotton boll: (a) destroyed by bollworms;
(b) a fully mature cotton boll**

- Pest resistant plants – decrease use of pesticide
- Bt cotton, Bt corn, rice, tomatoe, potato, soyabean, etc
- Bt toxin – endotoxin in bacterium **Bacillus thuringiensis** (inactive precursor form)
- Bt toxin gene has been cloned from bacteria and expressed in plants to provide resistance to insects, without need for insecticide (bio-pesticides)
- Bt cotton some strains of *B.thruingiensis* produce protein's that killed certain insect (lepidopterans, coleopterans, dipterans)
- Form's proteins crystals, which contains toxic insecticidal protein
- This toxin does not kill bacillus because it exists as inactive protoxins but once an insect ingest this, it is converted into active form due to alkaline pH of gut which solubilise crystals

Bt TOXIN

- ✓ Choice of genes - depends upon crop and the targeted pests (these toxins are insects – group specific)
- ✓ Genes cryIAc and cryIIAb – control cotton bollworms
- ✓ cryIAb - controls corn borer

➤ **Nematode Resistant Plant by RNA i :- by Fire & Mello**

In Nematode -

(*Meloidogyne incognita*) - Infects roots of Tobacco

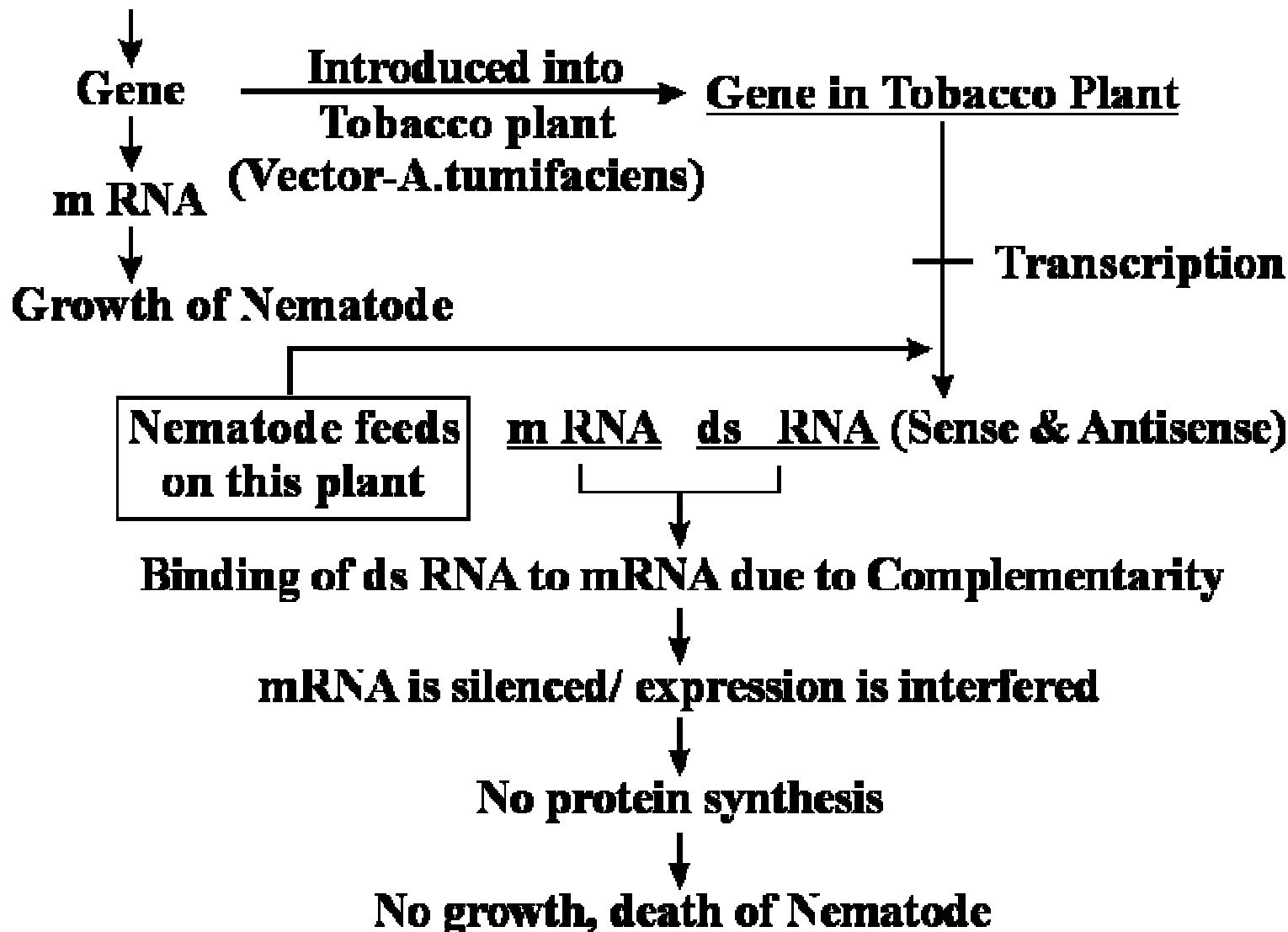
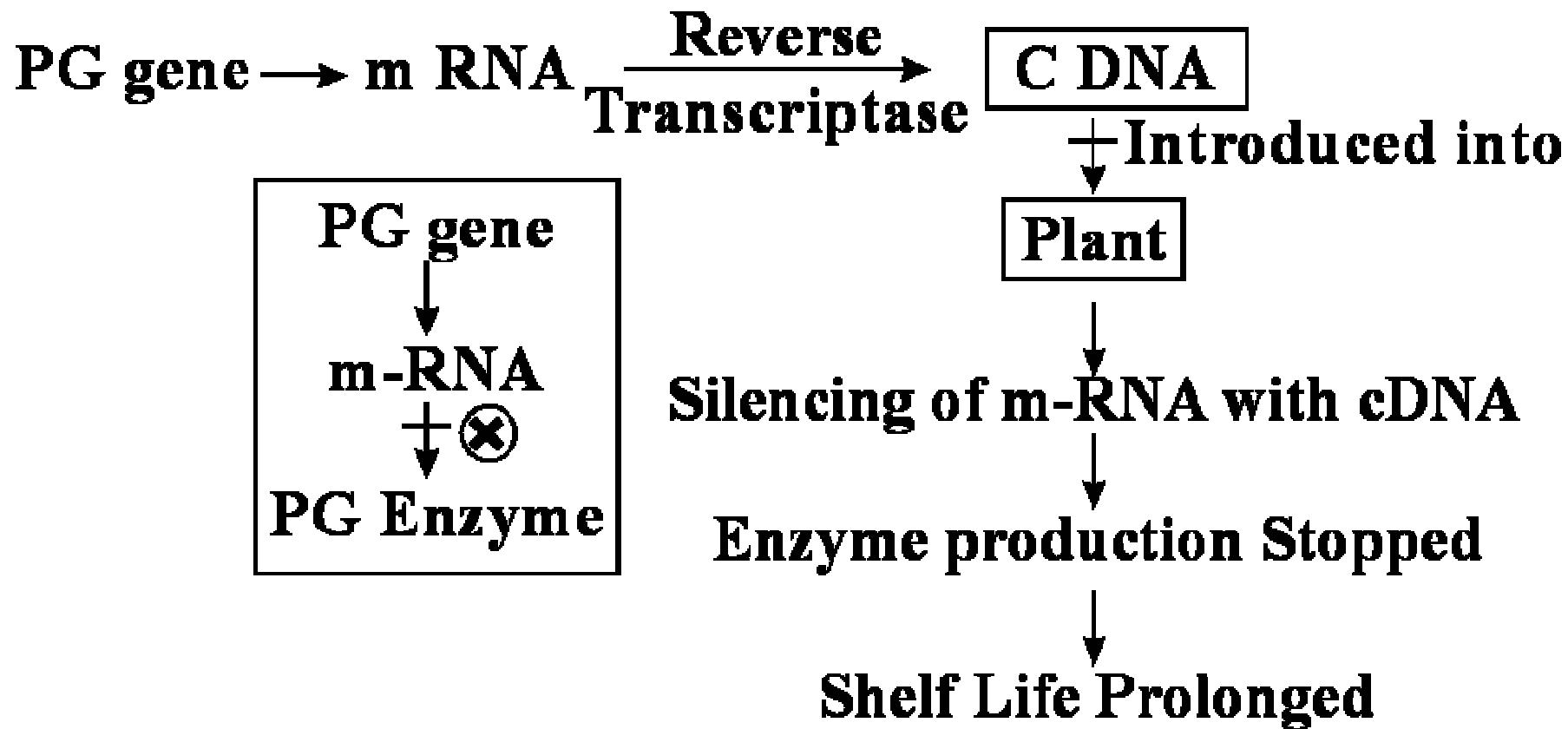




Fig: Host plant-generated dsRNA triggers protection against nematode infestation:
(a) Roots of a typical control plants; (b) transgenic plant roots 5 days after deliberate
infection of nematode but protected through novel mechanism

- Flavr Savr Tomatoes/ Mac Gregor tomatoes
 - ✓ Post harvest Losses/Delayed Fruit Ripening
 - ✓ Enzyme responsible for ripening of tomatoes – “Pectinase” (Gene : PG/ Polygalacturonase)



TRANSGENIC ANIMALS :

- They have their DNA manipulated to posses and express an extra- foreign gene
- 95% are mice (rats, rabbits, pigs, sheep, cows, fish)

Advantages-

1. Normal physiology and development eg. Study of complex factors involved in growth like insulin – like growth factor
2. Study of disease – models exists for cancer, cystic fibrosis, rheumatoid arthritis, alzheimer's
3. Vaccine safety – Transgenic mice to test safety of polio vaccine (could replace use of monkeys, if successful)

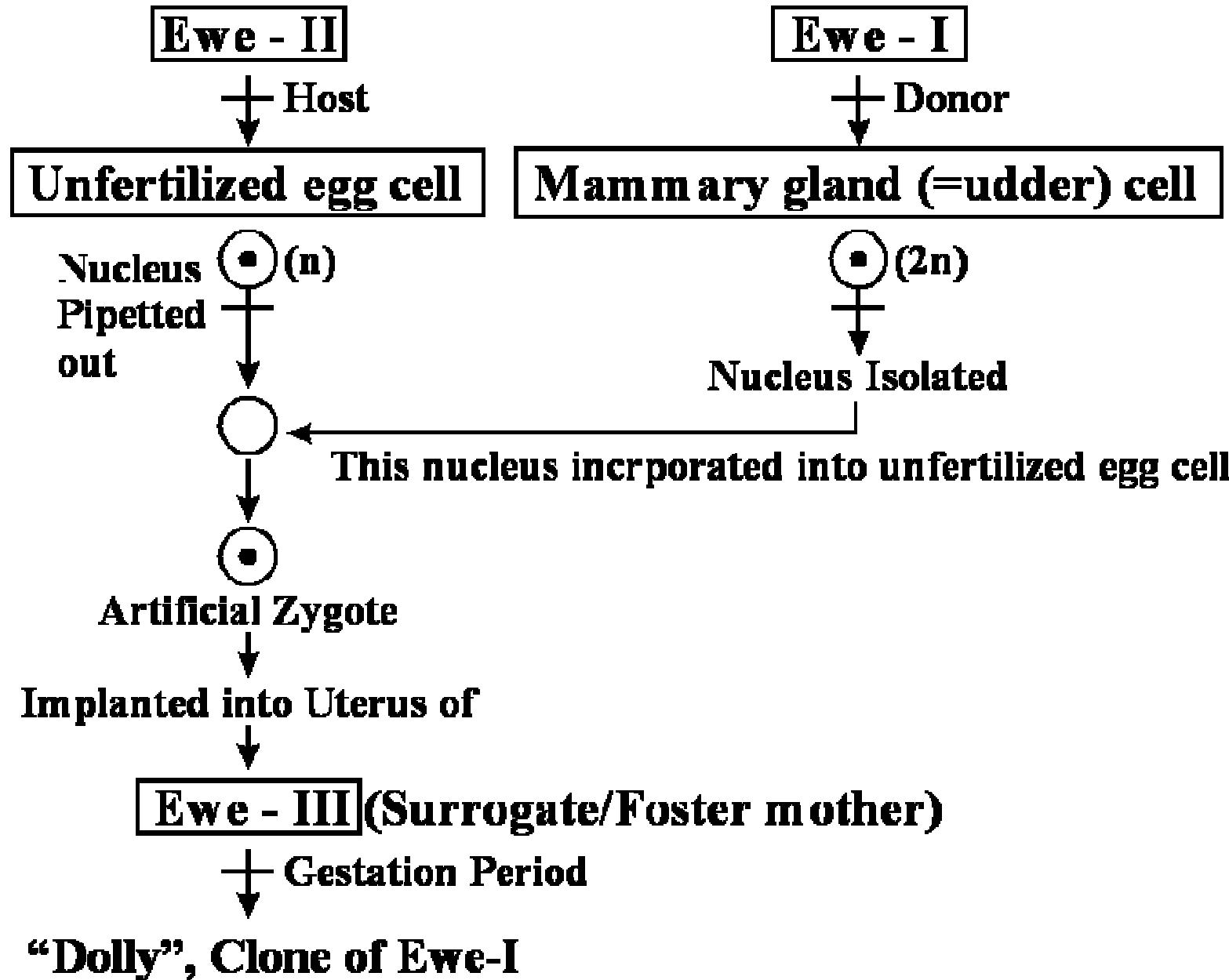
4. Biological products

- ✓ Human protein (α -1-antitrypsin) to treat emphysema
- ✓ Treatment of phenylketonuria (PKU) and Cystic fibrosis
- ✓ In 1997, first transgenic cow, Rosie – produce human protein enriched milk (2.4gms/litre), contained human alpha – lactalbumin (nutritionally more balanced product for human babies than natural cow milk)

5. Chemical safety testing /toxicity testing

- First successful mammalian clone
- Dolly sheep, created by Ian Wilmut et al at Roseline – Franklin Research Institute, Scotland
- First cloned monkey – ANDi (green fluorescent protein gene inserted)
- Ethical issues:
- Bioethics- set of standard to regulate our activities in relation to biological world can have unpredictable results when GMO are introduced into ecosystem
- GEAC (Genetic Engineering Approval Committee) by Indian government – make decision regarding validity of GM research, safety of introducing GMO for public services
- Biopatents – novelty, non-obviousness and utility
- 2 lakh varieties of rice in India alone (27 of basmati)

Dolly, the sheep :-



- In 1997, an American company got patent on basmati (Indian basmati was crossed with semi- dwarf varieties)
- Turmeric/neem
- If not vigilant, others may encash our rich legacy
- Biopiracy - use of Bio- resources by multinational companies and other organizations without proper authorization from the countries and people concerned without compensatory payment
- Industrilised nations – Rich financially, poor in biodiversity and traditional knowledge (developing and under-developed world – vise a versa)
- Indian parliament recently cleared second amendment of the Indian patents bill, that takes such issues into consideration, including patent terms emergency provisions and research and development initiative
- Bioweapon / Biowar

Genetically Engineered Microbes and their applications

Microbe	Application
<i>Bacillus thuringiensis</i>	Production of Bt toxin
<i>Escherichia coli</i>	Production of human insulin, growth hormone, interferon, etc.
<i>Pseudomonas fluorescens</i>	Prevention of frost damage to plants
<i>Pseudomonas putida</i>	Scavenging of oil spills by digesting hydrocarbons of crude oil
<i>Rhizobium meliloti</i>	Nitrogen fixation by 'nif' gene in cereal crops